

Award Number: W81XWH-11-1-0806

TITLE: Chronic Pain Following Spinal Cord Injury: The Role of Immunogenetics and Time of Injury Pain Treatment

PRINCIPAL INVESTIGATOR: Dr. Mark Hutchinson

CONTRACTING ORGANIZATION: University of Adelaide
Adelaide, AU 5000

REPORT DATE: October 2016

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE October 2016		2. REPORT TYPE Annual		3. DATES COVERED 30-Sep-2015 – 29Sep2016	
4. TITLE AND SUBTITLE Chronic Pain Following Spinal Cord Injury: The Role of Immunogenetics and Time of Injury Pain Treatment				5a. CONTRACT NUMBER W81XWH-11-1-0806	
				5b. GRANT NUMBER SC100231	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Dr. Mark Hutchinson, Dr. Janet Collier, Dr. Jillian Clark, Dr. Ruth Marshall Prof James Middleton Ms Vicky Staikopoulos Ms Melanie Alvaro, Ms. Kathy Heyman E-Mail: mark.hutchinson@adelaide.edu.au				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Adelaide, North Terrace, Adelaide, South Australia 5005, Australia.				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES See associated EWOFF in Oct 2016					
14. ABSTRACT We are in the 5th year of our research program into the immunogenetics and drug exposure factors that contribute to chronic pain following spinal cord injury. Recruitment for Study 1 has continued to be slow. However, we have continued to progress with the expansion of Study 1 recruitment sites by broadening our collaborative network. Owing to the delay in recruitment an extension without funds request has recently been submitted as has a revised statement of work.					
15. SUBJECT TERMS Spinal Cord Injury, Immunogenetics, Chronic pain, Opioids					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	14	19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
1. Introduction.....	
2. Keywords.....	
3. Accomplishments.....	
4. Impact.....	
5. Changes/Problems.....	
6. Products.....	
7. Participants & Other Collaborating Organizations.....	
8. Special Reporting Requirements.....	
9. Appendices.....	

INTRODUCTION

Spinal cord injury results in significant trauma and inflammation originating at the site of injury as well as from various systemic anatomical compartments. This inflammatory event provides both beneficial activation of repair and clearance systems, but also creates long-term detrimental consequences such as chronic pain. Chronic pain develops after spinal cord injury in more than 65% of the clinical population¹. However, the reasons why some patients develop chronic pain and others do not remains unknown. Chronic neuropathic pain elicits a number of changes in the activity, properties and transmitter content of pain-pathway neurons². This central sensitization to nociceptive stimuli culminates in profound debilitating pain that serves no adaptive purpose for the sufferer. It is now established that spinal inflammatory events resulting from numerous stimuli initiate and maintain chronic neuropathic pain conditions³ and may result from a dysregulation of the spinal immunocompetent cells, glia, and their up regulation of pronociceptive (pain) systems. A key event in the initiation of this inflammatory response is the activation of the innate immune system pattern recognition receptor, Toll Like Receptor 4 (TLR4)^{4,5}. TLR4 is able to detect the presence of endogenous danger molecular patterns, resulting in the activation of an inflammatory cascade that results in the expression and release of a myriad of inflammatory signals such as proinflammatory cytokines, chemokines, prostaglandins, reactive oxygen species and nitric oxide. Importantly, these same proinflammatory molecular signals also elicit a pronociceptive, or painful, response that contributes indelibly to the chronic pain state⁵. The prototypic opioid, morphine, is capable of TLR4-mediated proinflammation⁶⁻⁸. As such, exposure to morphine at the time of injury is hypothesised result in exacerbated proinflammation and hence produce long-term consequences for the pain susceptibility of the individual. In addition, the immune genes that encode these key inflammatory mediators are highly polymorphic. Hence, an individual may have a genetic predisposition to over respond in a proinflammatory fashion to the spinal cord injury, and/or to experience inflammation in response to opioid exposure. Critically, this genetic variability may significantly impact the long-term health and quality of life of the individual. Thus both genetics and drug exposure at the time of injury may be contributing factors individually and/or interactively that may lead some individuals to develop chronic pain following injury, or may protect others from developing pain pathology. Hence, this project is investigating the impact of both pharmacological agents and genetic variability on the occurrence of chronic pain following spinal cord injury.

KEYWORDS

Pain, spinal cord injury, opioid, glia, innate immunology, single nucleotide polymorphism, genetics

ACCOMPLISHMENTS

The research management team (Prof Hutchinson, Dr Collier & Dr Clarke), laboratory research (Vicky Staikopoulos) and the clinical unit team (Kathy Heyman & Francesca Zappia) met between fortnightly to monthly during year 4 of the research project to ensure administrative progress. During this year Dr Collier and Francesca Zappia meet weekly for 6 months to drive recruitment and facilitate the addition of a new site Austin Health in Victoria Australia.

Our professional clinical research team has continued in their efforts of subject recruitment, screening and testing. They have continued to liaise with the spinal cord injury unit at site 1 (Royal Adelaide Hospital) to get new spinal cord injury patient information. Ms Vicky Staikopoulos is our highly experienced technical research associate who is working on the laboratory side of the team to process DNA samples and receive data back from our analysis center. Vicky also has the leadership role in managing the human ethics for our Royal North Shore Hospital (RNSH) recruitment site. This continues to be a very challenging role owing to significant administrative delays at the RNSH. Dr Collier and Francesca Zappia have taken a joint role in preparing our subject mail-outs and direct phone contact.

Ms Francesca Zappia (nee Alvaro) and Ms Kathy Heyman form the hands on part of our clinical team who are engaged at the Hospital and the Rehabilitation Centre. As highlighted previously we expanded our recruitment sites during year 4 to RNSH to facilitate our expanded subject recruitment drive. Francesca and Kathy liaise with Dr Marshall and Dr Clarke to coordinate the subject recruitment and consenting in South Australia; and with Prof Middleton for activities at the RNSH. Kathy is a registered nurse and Francesca has many years of clinical trials experience. Kathy as a registered nurse has taken a leadership role in reviewing the participants medical records.

This staffing arrangement has continued to work well during year 5 to ensure a balance of strict patient information confidentiality at the hospital side and timely outcomes at the lab side. All staff and investigators have completed NIH Human Research training and their details and credentials have been passed onto the Human Research Protection Office.

STUDY 1

Human research ethics for Study 1 Site 1 (Royal Adelaide Hospital : approval no. 111008) and approved by the Human Research Protection Office (16986.1a) has been maintained with required periodic RAH HREC reporting and associated HRPO notifications. The Royal Adelaide Hospital Ethics Committee has approved the addition a new site, Site 3 Austin Health in Victoria Australia. The Research Governance Office of the Austin Health site is currently reviewing the submission. Study 1 Site 2 ethics has maintained approval (Royal North Shore Hospital, in New South Wales, Australia: approval no. SSA/14/HAWKE/71; 1311-372M) and included under HRPO 16986.1b.

The clinical team at Site 1 (Hampstead Rehabilitation Centre, Royal Adelaide Hospital) has identified over 850 patients in their database who fulfill the inclusion criteria for Study 1. They have been processing 20-30 recruitment and questionnaire/sample mail-outs per month. To date, recruitment from the RAH site remains considerably lower than anticipated as detailed in our request for an extension without funds and revised statement of work documentation. We are continuing to make follow-up phone calls to all participants who have been sent study invitations letters but have not responded. To date we have recruited 61 people at the RAH site 1 and

enrolled 47.

It is clear from the dialogue with the SCI population that other concurrent trials, such as the very large cold therapy trial, are perceived (but not mandated) as an exclusion from all other clinical trials such as ours. We are continuing to work with the population to improve the communication of these key messages.

Site 2 at RNSH has been sending out recruitment information packs with 740 packs sent to date. Once again, the response rate to this call has been dismally low, with only 30 patients recruited. This is an unprecedentedly low response rate.

Buccal sample processing and DNA genotyping has continued as the samples have been returned by the participants. This data has been entered into our database, along with the audit entries from the participant's medical histories.

Parallel to these efforts in the spinal cord injury patient population we have expanded the collection of the DNA samples from the healthy control reference sample population.

To grow out recruitment sites to fulfill the 225 subjects required we have begun the ethics and governance approval process at the Austin in Victoria which has access to a similar number of patients as the NSW site. As such we anticipate, despite the low recruitment rates to be approaching 150-200 patients by July-Sept in 2017.

COLLABORATION

Despite these set backs, the process of drawing together the broader range of clinicians, health workers and life scientists around our project has triggered a range of new and very exciting collaborative projects outside the remit of this funding.

The clinical trial of Dr Clarke and Prof Hutchinson on a novel blood tests with predictive ability of motor recovery upon discharge continues through the collaboration built upon the \$42M Australian Research Council Centre of Excellence for Nanoscale BioPhotonics and the ability to use advanced BioPhotonic imaging and sensing to provide point of care devices in the future.

Dr Coller and Prof Hutchinson have continued to explore the immunogenetic origins of pain susceptibility with Prof Somogyi and Dr Barratt. This collaboration resulted in a non-project related publications in pain and anesthesia related areas.

- Somogyi AA, Sia AT, Tan EC, Coller JK, Hutchinson MR, Barratt DT.
Ethnicity-dependent influence of innate immune genetic markers on morphine PCA requirements and adverse effects in postoperative pain. *Pain*. 2016 Nov;157(11):2458-2466.
- Li Y, Jackson KA, Slon B, Hardy JR, Franco M, William L, Poon P, Coller JK, Hutchinson MR, Currow DC, Somogyi AA. CYP2B6*6 allele and age substantially reduce steady-state ketamine clearance in chronic pain patients: impact on adverse effects. *Br J Clin Pharmacol*. 2015 Aug;80(2):276-84. doi: 10.1111/bcp.12614.

IMPACT

The inherent nature of the study is that all our results are back ended and as such the scientific impact will be associated with this long timeline.

CHANGES/PROBLEMS

As outlined above, recruitment has been unprecedentedly slow. As such, we have sought and had approved a no cost extension and a revision of the statement of work.

Included below is a copy of the revised statement of work

Revised Statement of work for W81XWH-11-1-0806

All work will be carried out at the University of Adelaide, Medical School (L5 North Wing) Frome Rd, and the Pain and Anaesthesia Research Clinic, Royal Adelaide Hospital, North Tce, Adelaide, South Australia.

SPECIFIC AIMS 1

TASK 1 – STUDY 1

This task involves recruiting 225 spinal cord injury (SCI) patients and 450 healthy individuals for immunogenetic analysis and collation of demographic parameters and clinical outcome data.

1a. Submit human ethics protocol and receive approval (months 1-6, University of Adelaide Human Research Ethics Committee meets every 2 months)

COMPLETED APROVAL

Added RNSH as a recruitment site

We are awaiting the approval of a Victorian recruiting site Austin Health to help meet recruitment goals.

1b. Recruitment of study participants and nation-wide mail out of buccal swabs (commercially available foam-tip swab) for collection of cell samples. Genomic DNA will be extracted from the brushes upon receipt in Adelaide using the modified manufacturer protocol. Concentration and purity of isolated DNA will be determined using standard techniques measuring absorption at 260 and 280 nm on a spectrophotometer. DNA will be diluted to specific concentrations using a QIAgility pipetting robot and shipped in batches of 500 to AGRF for genotyping (turn-around of 4-6 weeks), with the remaining sample archived at -80°C.

ONGOING

1c. Demographic data and frequency of clinical outcome measures of pain and current opioid use will be collated from clinical case notes and study information report forms and entered for each participant into a computer database, with appropriate coding for use in bioinformatic analysis.

ONGOING

1d. Bioinformatic analysis: Raw data for individual SNPs will be analysed with regard to allele and genotype Data will use initial data batches to design a pipeline for multiple regression analysis that will include demographic factors and clinical outcome data collected in task 1c as covariates, with α -levels adjusted with false discovery rate calculations to account for multiple testing. As more data becomes available this analysis will be refined and updated.

ONGOING

1f. Final conclusions from study outcomes completed and presented at an appropriate national or international scientific meeting, and milestone manuscripts prepared for publication, in 18 months (Dec 2018).

ONGOING

PRODUCTS

Not applicable

PARTICIPANTS AND OTHER COLLABORATING ORGANISATIONS

Roster for past year

Prof Mark Hutchinson (University of Adelaide)

Dr Janet Coller (University of Adelaide)

Dr Jillian Clarke (University of Adelaide)

Dr Ruth Marshal (Royal Adelaide Hospital)

Dr James Middleton (Royal North Shore Hospital)

Dr Paul Rolan (University of Adelaide)

Ms Vicky Staikopoulos (University of Adelaide)

Mrs Francesca Zappia (University of Adelaide)

Mrs Kathy Hayman (University of Adelaide)

Anticipated growth of roster – subject to approvals

Prof Andrew Nunn (Austin Health/Monash)

SPECIAL REPORTING REQUIRMENTS

Not applicable

APPENDICES

1. Siddall, P.J. & Loeser, J.D. (2001) Pain following spinal cord injury. *Spinal Cord* 39, 63-73
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11402361
2. Roza, C., Laird, J.M., Souslova, V., Wood, J.N. & Cervero, F. (2003) The tetrodotoxin-resistant Na⁺ channel Nav1.8 is essential for the expression of spontaneous activity in damaged sensory axons of mice. *The Journal of Physiology* 550, 921-926
3. Milligan, E.D. & Linda Watkins, L.R. (2009) Pathological and protective roles of glia in chronic pain. *Nat. Rev. Neurosci.* 10, 23-36
4. Tanga, F., Natile-McMenemy, N. & Deleo, J.A. (2005) The CNS role of Toll-like receptor 4 in innate neuroimmunity and painful neuropathy. *Proc. Natl. Acad. Sci. U. S. A.*
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=15809417
5. Hutchinson, M.R., Zhang, Y., Brown, K., Coats, B.D., et al. (2008) Non-stereoselective reversal of neuropathic pain by naloxone and naltrexone: involvement of toll-like receptor 4 (TLR4). *Eur. J. Neurosci.* 28, 20-29
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=18662331
6. Hutchinson, M., Zhang, Y., Shridhar, M., Evans, J., et al. (2010) Evidence that opioids may have toll-like receptor 4 and MD-2 effects. *Brain. Behav. Immun.* 24, 83-95
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=19679181
7. Lewis, S., Hutchinson, M.R., Rezvani, N., Loram, L., et al. (2009) Evidence that intrathecal morphine-3-glucuronide may cause pain enhancement via toll-like receptor 4/MD-2 and interleukin-1 β . *Neuroscience* 4
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=19833175
8. Watkins, L.R., Hutchinson, M.R., Rice, K.C. & Maier, S.F. (2009) The "toll" of opioid-induced glial activation: improving the clinical efficacy of opioids by targeting glia. *Trends Pharmacol. Sci.* 30, 581-591
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=19762094